Anti-MUC1 Antibody [SN06-80]

ET1611-14

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 122 kDa
Clone number:	SN06-80

Description: Mucin 1, cell surface associated (MUC1), also called polymorphic epithelial mucin (PEM) or epithelial membrane antigen or EMA, is a mucin encoded by the MUC1 gene in humans. MUC1 is a glycoprotein with extensive O-linked glycosylation of its extracellular domain. Mucins line the apical surface of epithelial cells in the lungs, stomach, intestines, eyes and several other organs. Mucins protect the body from infection by pathogen binding to oligosaccharides in the extracellular domain, preventing the pathogen from reaching the cell surface. Overexpression of MUC1 is often associated with colon, breast, ovarian, lung and pancreatic cancers.

Immunogen: Synthetic peptide within Human MUC1 aa 1,206-1,255 / 1,255.

Positive control: HeLa cell lysate, T-47D cell lysate, mouse lung tissue lysate, rat lung tissue lysate, HeLa, B16F1, human lung carcinoma, human endometrial carcinoma, human kidney tissue.

Subcellular location: Apical cell membrane, Secreted, Cell membrane, Cytoplasm, Nucleus.

Database links: SwissProt: P15941 Human | Q02496 Mouse
Entrez Gene: 24571 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:500
IF-Tissue	1:500
IHC-P	1:50-1:1,000
FC	1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

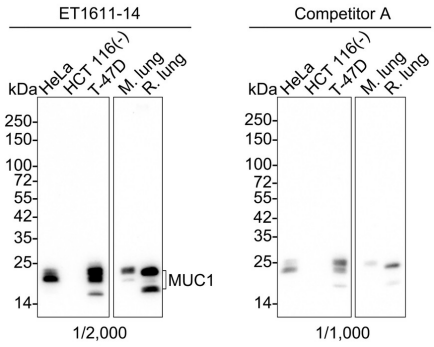

Fig1: Western blot analysis of MUC1 on different lysates with Rabbit anti-MUC1 antibody (ET1611-14) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HCT 116 cell lysate (negative) (20 µg/Lane)
Lane 3: T-47D cell lysate (20 µg/Lane)
Lane 4: Mouse lung tissue lysate (40 µg/Lane)
Lane 5: Rat lung tissue lysate (40 µg/Lane)

Predicted band size: 122 kDa
Observed band size: 17~24 kDa

Exposure time: 3 minutes 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-14) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

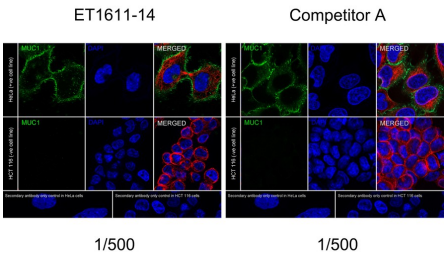

Fig2: Immunocytochemistry analysis of HeLa (positive) and HCT 116 (negative) labeling MUC1 with Rabbit anti-MUC1 antibody (ET1611-14) at 1/500 dilution and competitor's antibody at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-MUC1 antibody (ET1611-14) at 1/500 dilution and competitor's antibody at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

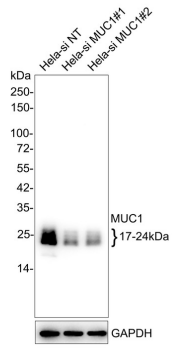

Fig3: Western blot analysis of MUC1 on different lysates with Rabbit anti-MUC1 antibody (ET1611-14) at 1/2,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si MUC1#1 cell lysate
Lane 3: Hela-si MUC1#2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 122 kDa
Observed band size: 17~24 kDa

Exposure time: 10 seconds;

4-20% SDS-PAGE gel.

ET1611-14 was shown to specifically react with MUC1 in Hela-si NT cells. Weakened bands were observed when Hela-si MUC1 sample were tested. Hela-si NT and Hela-si MUC1 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1611-14, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

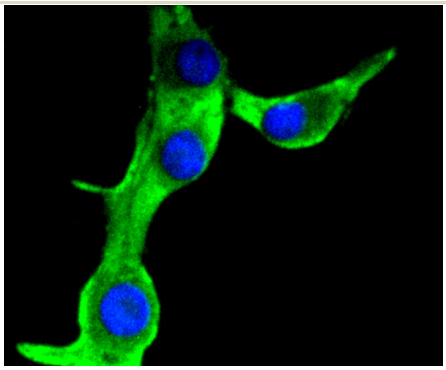

Fig4: ICC staining of MUC1 in B16F1 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-14, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

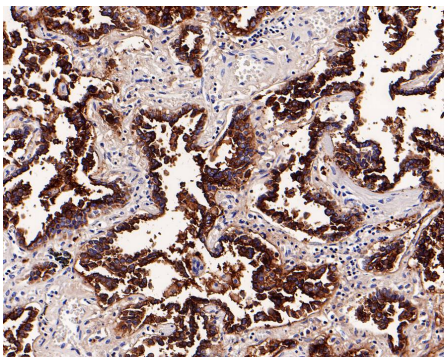

Fig5: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

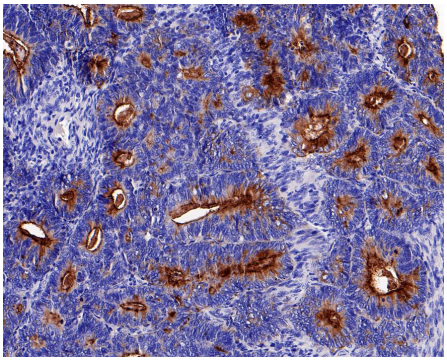

Fig6: Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

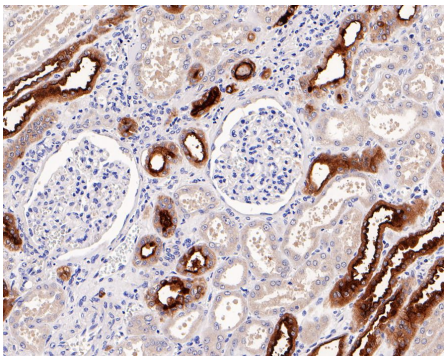

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

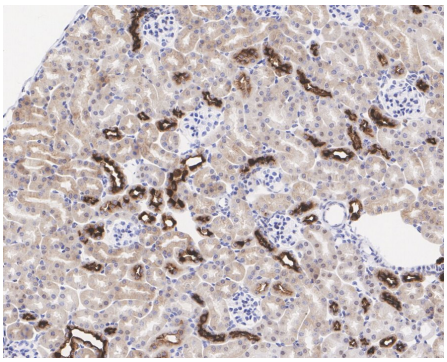

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

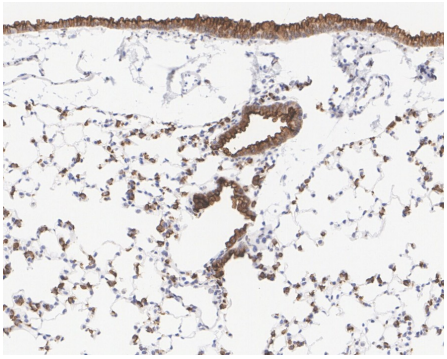

Fig9: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

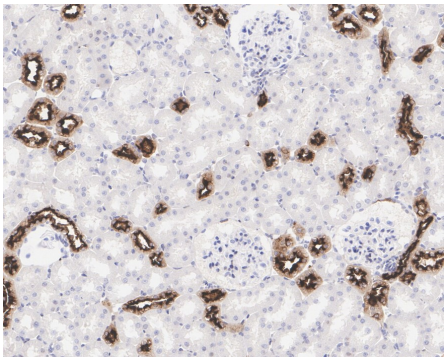

Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-MUC1 antibody (ET1611-14) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-14) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

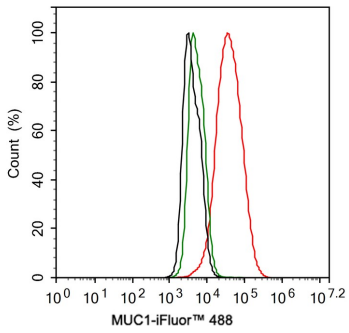

Fig11: Flow cytometric analysis of HeLa cells labeling MUC1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1611-14, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

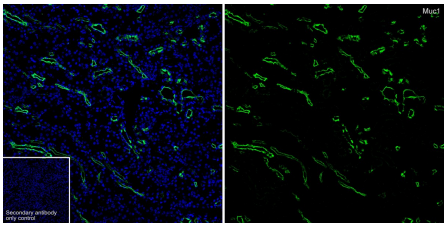

Fig12: Application: IF-Tissue

Species: Mouse

Site: kidney

Sample: Paraffin-embedded section

Antibody concentration: 1/500

Background References

1. Gorges TM et al. Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. BMC Cancer 12:178 (2012).
2. Williams MA et al. Deletion of the mucin-like molecule muc1 enhances dendritic cell activation in response to toll-like receptor ligands. J Innate Immun 2:123-43 (2010).